

Claims

1. A protein having a covalently bonded puromycin tag, said tag being positioned at the C-terminal end of said protein.

2. The protein of claim 1, wherein said tag is a small molecule.

5 3. The protein of claim 2, wherein said small molecule is biotin.

4. The protein of claim 1, wherein said tag is a detectable label.

5. The protein of claim 4, wherein said detectable label is fluorescein, rhodamine, or BODIPY, or derivatives thereof.

6. The protein of claim 1, wherein said tag is a functional group.

10 7. The protein of claim 1, wherein said protein has a first functional group and said tag is a second functional group and wherein said first functional group has a reactivity orthogonal to the reactivity of said second functional group.

8. The protein of claim 1, wherein said tag is a tether for attachment to a solid support.

15 9. The protein of claim 8, wherein said solid support is a column, bead, or chip.

10. The protein of claim 1, wherein said tag is one member of a specific binding pair.

11. The protein of claim 10, wherein said tag is a phenyl diboronic acid derivative.

5 12. The protein of claim 1, wherein said puromycin tag further comprises a nucleotide sequence positioned between said tag and said puromycin.

13. The protein of claim 12, wherein said nucleotide sequence is between about 1-200 nucleotides in length.

10 Sub A12 14. A method for C-terminal protein tagging, comprising
(a) providing a nucleic acid sequence encoding said protein;
(b) translating said sequence under conditions in which translation stalls at the 3' end of said sequence, forming a stalled translation complex; and
(c) contacting said stalled translation complex with a puromycin tag under conditions in which said puromycin tag is covalently bonded to the C-terminus of said protein.
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15. The method of claim 14, wherein said tag is attached to the 5'-hydroxy group of said puromycin.

16. The method of claim 15, wherein said tag is attached to the 5'-hydroxy group of said puromycin through a phosphate group.

17. The method of claim 14, wherein said nucleic acid sequence encoding said protein contains no stop codons.

Sub A13

18. The method of claim 14, wherein said translation step is carried out in the substantial absence of at least one translation release factor.

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19. The method of claim 14, wherein the 3'-end of said nucleic acid sequence encoding said protein is covalently linked to a DNA oligomer.

Sub A14

20. The method of claim 14, wherein said tag is a small molecule.

21. The method of claim 20, wherein said small molecule is biotin.

Sub A15

22. The method of claim 14, wherein said tag is a detectable label.

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23. The method of claim 22, wherein said detectable label is fluorescein, rhodamine, or BODIPY, or a derivative thereof.

Sub A16

24. The method of claim 14, wherein said tag is a functional group.

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25. The method of claim 14, wherein said protein has a first functional group and said tag is a second functional group and wherein said first functional group has a reactivity orthogonal to the reactivity of said second functional group.

26. The method of claim 14, wherein said tag is a tether for attachment

to a solid support.

27. The method of claim 26, wherein said solid support is a column, bead, or chip.

A17 28. The method of claim 14, wherein said tag is one member of a
5 specific binding pair.

29. The method of claim 28, wherein said tag is a phenyl diboronic acid derivative.

30. The method of claim 14, wherein said puromycin tag further comprises a nucleotide sequence positioned between said tag and said puromycin.

31. The method of claim 30, wherein said nucleotide sequence is between about 1-200 nucleotides in length.